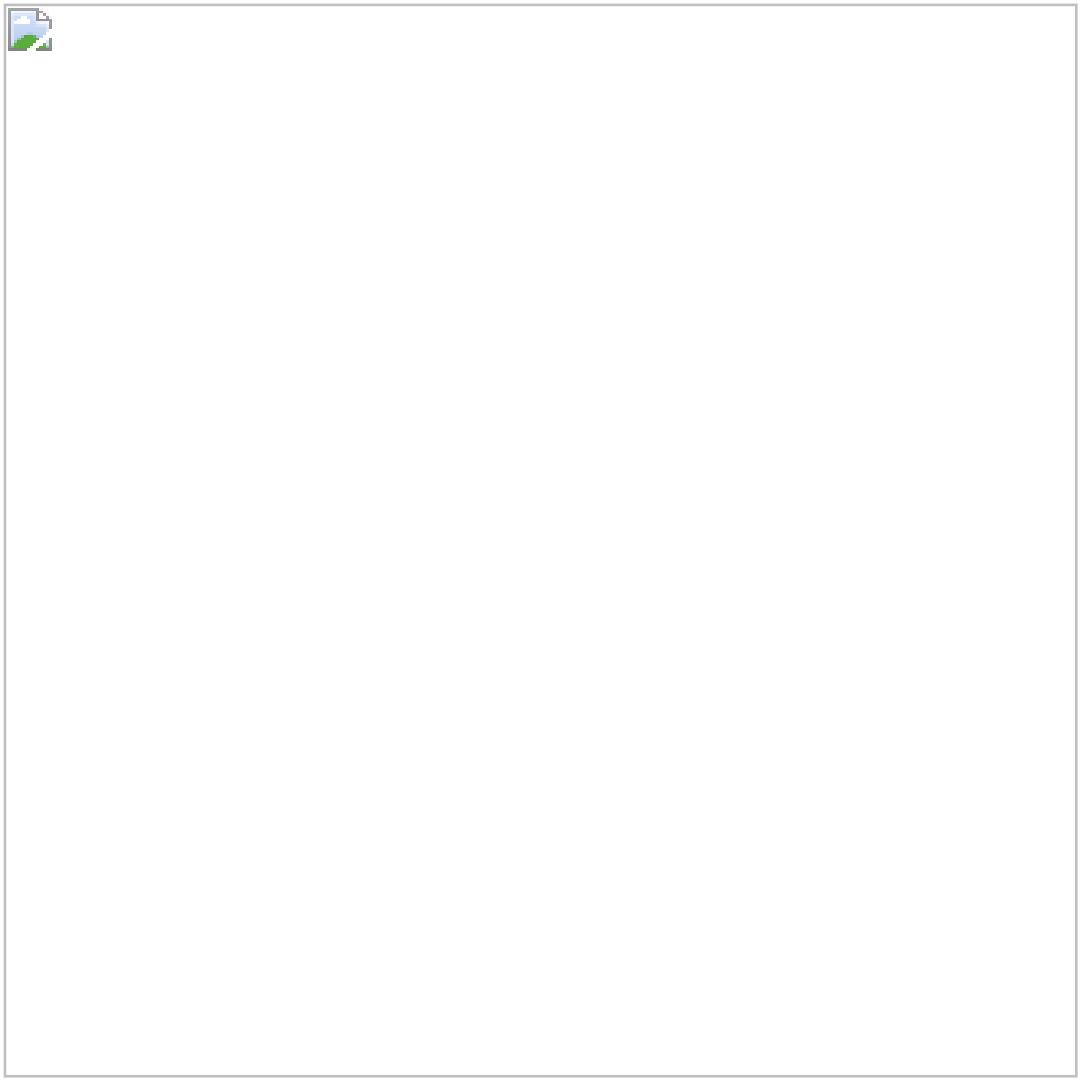

Fungus-Specific SSR Markers in the Antarctic Lichens Usnea antarctica and U. aurantiacoatra (Parmeliaceae, Ascomycota)

Authors: Lagostina, Elisa, Grande, Francesco Dal, Ott, Sieglinde, and Printzen, Christian

Source: Applications in Plant Sciences, 5(9)

Published By: Botanical Society of America

URL: https://doi.org/10.3732/apps.1700054

Applications in Plant Sciences 2017 5(9): 1700054

PRIMER NOTE

FUNGUS-SPECIFIC SSR MARKERS IN THE ANTARCTIC LICHENS *USNEA ANTARCTICA* AND *U. AURANTIACOATRA* (PARMELIACEAE, ASCOMYCOTA)[1]

ELISA LAGOSTINA[2,5], FRANCESCO DAL GRANDE[3], SIEGLINDE OTT[4], AND CHRISTIAN PRINTZEN[2]

[2]Department of Botany and Molecular Evolution, Senckenberg Research Institute and Natural History Museum Frankfurt, Senckenberganlage 25, D-60325 Frankfurt am Main, Germany; [3]Senckenberg Biodiversity and Climate Research Centre (BiK-F), Senckenberganlage 25, D-60325 Frankfurt am Main, Germany; and [4]Institute of Botany, Heinrich-Heine-University (HHU), Düsseldorf, Germany

- *Premise of the study: Usnea antarctica* and *U. aurantiacoatra* (Parmeliaceae) are common lichens in the maritime Antarctic. These species share the same habitats on King George Island (South Shetland Islands, Antarctica) and are distinguishable based on reproductive strategies.
- *Methods and Results:* We developed 23 fungus-specific simple sequence repeat (SSR) markers that cross-amplify between the two species. We used a low-coverage genome-skimming approach on one sample of each species to identify SSR repeats in the two species. Primers were designed for 3–4-bp repeats, and only the loci common to both species were selected for further analyses. Seventy-seven samples of the two species were selected to assess fungal specificity, genetic variability, and linkage of the markers. In addition, we tested cross-amplification in other *Usnea* species.
- *Conclusions:* The 23 newly designed SSR markers are suitable for population genetic and phylogeographic studies of *Usnea* species.

Key words: Antarctic lichens; microsatellites; Parmeliaceae; *Usnea antarctica*; *Usnea aurantiacoatra*.

Lichens constitute an important component of Antarctic terrestrial biota. The most common lichen species in the maritime Antarctic are *Usnea aurantiacoatra* (Jacq.) Bory and *U. antarctica* Du Rietz (Lecanoromycetes, Parmeliaceae). The two species occupy similar habitats but show different dispersal strategies, morphology, and distribution. *Usnea antarctica* usually propagates asexually by so-called soredia, while *U. aurantiacoatra* has apothecia and its thalli are usually larger. *Usnea antarctica* is reported to be circumpolar and to have the widest ecological amplitude and distribution of any Antarctic macrolichen (Øvstedal & Lewis Smith, 2001), while *U. aurantiacoatra* is absent from the continental Antarctic. Phylogenetic analysis, performed on several Southern Hemisphere *Usnea* Dill. ex Adans. species, showed that the two species are closely related, and they are considered a single group. Some authors have suggested that the two species might constitute a species pair, in which *U. aurantiacoatra* represents the fertile and *U. antarctica* the sterile counterpart, but phylogenetic studies indicated that they could be conspecific (Wirtz et al., 2012). Here, we developed highly variable microsatellite loci to better understand the genetic differences between the two lichen species and to investigate gene flow between maritime Antarctica and South America.

METHODS AND RESULTS

Total genomic DNA was extracted from a thallus of *U. antarctica* from population C1 and from one thallus of *U. aurantiacoatra* from a population that was not included in the later primer design (Appendix 1) from King George Island, Antarctica, in the austral summer of 2015/2016. Twenty milligrams of thallus were pretreated with acetone to remove secondary metabolites and ground with liquid nitrogen using a sterilized mortar and pestle. DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) protocol (Cubero and Crespo, 2002). Paired-end libraries (300 bp) were prepared and sequenced on a MiSeq version 3 (2 × 300-bp) Illumina sequencer (LGC Genomics GmbH, Berlin, Germany).

We obtained 3,098,758 raw reads for *U. antarctica* and 1,755,882 for *U. aurantiacoatra*. Raw data were adapter-, quality- (PHREAD = 26), and length- (>150 bp) filtered using Trimmomatic version 0.35 (Bolger et al., 2014). Forward and reverse reads were assembled with Paired-End reAd mergeR (PEAR) software (Zhang et al., 2014). The resulting overlapping, paired, and singleton reads were then assembled with SPAdes version 3.9 (Nurk et al., 2013). Scaffolds were taxonomically binned using Metawatt (Strous et al., 2012). Scaffolds assigned to the phylum Ascomycota were used to search for mycobiont-specific microsatellite motifs. Simple sequence repeat (SSR) motifs repeated at least six times were searched in both genomes with the MIcroSAtellite Identification Tool (MISA) (Thiel et al., 2003), and primers were designed for 3–4-bp repeats using the Primer3 plugin in Geneious 10 (Kearse et al., 2012). A total of 150 SSRs were identified in the two species. Each repeat from one genome was BLASTed against the other genome. Due to the partial coverage of both

[1]Manuscript received 19 May 2017; revision accepted 4 July 2017.

The authors thank the staff of the Alfred Wegener Institute (AWI; Bremerhaven, Germany) and the Argentinian research station Carlini (KGI) for logistic support. This work was supported by the Deutsche Forschungsgemeinschaft (DFG) in the framework of the priority program "Antarctic Research with comparative investigations in Arctic ice areas" by grant PR 567/18-1.

[5]Author for correspondence: elisa.lagostina@senckenberg.de

doi:10.3732/apps.1700054

genomes, only 30 SSRs were found to share the same flanking regions in silico. We designed primers for these 30 markers and tested them on four samples of *U. antarctica* and *U. aurantiacoatra* in single touchdown PCR reactions. After sequencing we selected 23 markers (Table 1) with perfectly matching flanking regions to be tested in multiplex reactions and on algal pure cultures to confirm that they were fungal specific. The remaining seven markers were excluded because of insertions and/or deletions in the flanking regions.

A fluorescent dye–associated tag was attached to the forward primers. Four different tails were selected to multiplex the reactions (FAM: GCCTCCCTCGCGCCA, VIC: GCCTTGCCAGCCCGC, NED: CAGGACCAGGCTACCGTG, PET: CGGAGAGCCGAGAGGTG) (Blacket et al., 2012). PCRs were performed in 25-µL reactions using illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare Life Sciences, Little Chalfont, Buckinghamshire, United Kingdom) according to the manufacturer's protocol with the following touchdown conditions: initial hot start at 94°C for 15 min; five cycles of 94°C for 45 s, 65°C for 45 s, 72°C for 45 s; five cycles of 94°C for 45 s, 60°C for 45 s, 72°C for 45 s; 10 cycles of 94°C for 45 s, 57°C for 45 s, 72°C for 45 s; 20 cycles of 94°C for 45 s, 55°C for 45 s, 72°C for 45 s; and final elongation at 72°C for 15 min. The PCR products were sent to Macrogen Europe (Amsterdam, The Netherlands) for sequencing to check the stability of the flanking regions in both species. To confirm that the chosen SSRs are indeed fungal loci, the primers were also tested on four axenic cultures of *Trebouxia jamesii* (Hildreth and Ahmadjian) Gärtner isolated from *U. antarctica*, *U. aurantiacoatra*, *U. lambii* (Imshaug) Wirtz & Lumbsch, and *U. trachycarpa* (Stirt.) Müll. Arg. (Appendix 1). The PCRs were performed under the same conditions as above, and no amplifications were observed.

After fungal specificity of the primers was confirmed, 77 samples from two populations of *U. antarctica* and *U. aurantiacoatra* (sampled in the same areas

TABLE 1. Characteristics of 23 fungus-specific SSR primers developed for *Usnea antarctica* and *U. aurantiacoatra.*[a]

Locus	Primer sequences (5′–3′)	Repeat motif	Tail[b]	Fluorescent dye	Allele size range (bp)[c]	No. of SSR repeats	*A*	Multiplex	GenBank accession no.
Ua1	F: GCATCTGGGCTCTTGGACTT	$(CTT)_n$	A	FAM	235–271	3–15	9	1	KY940723
	R: CATTTGCAGGCAGTCCATCG								
Ua2	F: GGGATAACTCGCTATGGCCC	$(CTT)_n$	B	VIC	256–283	6–15	9	1	KY940724
	R: ACACCCTGATCGATCAAACCA								
Ua3	F: AAGCACACGCAAAGCTTCAG	$(CTG)_n$	C	NED	248–275	4–13	6	1	KY940725
	R: CGGAGGTCTGAATGTCGGAG								
Ua4	F: CTTTCACTGTCCTGCCCTGT	$(CCT)_n$	D	PET	277–304	2–13	10	1	KY940726
	R: GAGACCCCGTGTCCAATCAG								
Ua5	F: GGAAGGGGAAGGGGAGAGAT	$(CTT)_n$	A	FAM	555–573	8–14	4	1	KY940727
	R: GGTGGGCAACTGGAATGGTA								
Ua6	F: TTGAGCCGCCACAAGAGATT	$(AAG)_n$	B	VIC	337–349	4–8	5	1	KY940728
	R: ATCGGCCAATTGATACCCCG								
Ua7	F: AAGACGGACATTCCACCACC	$(GGA)_n$	D	PET	552–573	2–9	5	1	KY940729
	R: ACCGCTCTGGCTACCTCTTA								
Ua8	F: AAGAAGCCAGCTTTGACGGA	$(TGT)_n$	A	FAM	393–402	6–9	4	1	KY940730
	R: GCTTGTCTCAGGCAGGATGA								
Ua9	F: AACGGAGCTTCCTTCCATTGA	$(CATC)_n$	A	FAM	285–297	8–11	4	2	KY940731
	R: ACAACACAGACAACCCCGAA								
Ua10	F: GACTTTACGGCCCACATCCA	$(AGA)_n$	B	VIC	284–314	4–14	8	2	KY940732
	R: TTTCCATGTGGCTTGGAGGG								
Ua11	F: TCGCATTATTCGTGCAAGCG	$(TATG)_n$	C	NED	254–274	5–10	6	2	KY940733
	R: GTAATATCCGCTCGCCCACA								
Ua12	F: AGGCGCTGTGTGAGAACC	$(CGAA)_n$	D	PET	232–256	3–9	6	2	KY940734
	R: AGCAAGACCAAGAAGGCGAG								
Ua13	F: CCAAGCCAACCTCAGACCAT	$(CAG)_n$	A	FAM	393–408	4–9	6	2	KY940735
	R: CGACGTCTCCTTCCATAGCC								
Ua14	F: GTCAGCCCATCTACCGTACG	$(GAA)_n$	B	VIC	386–419	5–16	8	2	KY940736
	R: TGGGTTGGGAAAGGAAGTGT								
Ua15	F: CGCAAACAGTACAACCGGAA	$(GCT)_n$	C	NED	341–347	5–7	3	2	KY940737
	R: GCCACAACAAAGGTGACGAC								
Ua16	F: GTTTGGAAGACCACCGGCTA	$(AGT)_n$	D	PET	334–355	10–17	6	2	KY940738
	R: CCAAGCACACCCTGACATCT								
Ua17	F: ATGACGTGCTGTAGGTGTGG	$(CTGGTA)_n$	A	FAM	403–415	4–6	3	3	KY940739
	R: GTGTCAAGTGTCGAGCAGGA								
Ua18	F: AGGGAGTTCTGCAGGGGATA	$(GAA)_n$	B	VIC	355–388	6–17	6	3	KY940740
	R: AGTGATTGATGCTCCGGTGG								
Ua19	F: AGCCATTTTTCCGAGGTCGT	$(GAC)_n$	C	NED	349–367	6–12	4	3	KY940741
	R: GCTTTGTTGCGCTTCACTGA								
Ua20	F: GATCACTCTTCGAGCTCCCG	$(AAGC)_n$	D	PET	405–421	6–10	5	3	KY940742
	R: CCAGAGTACCTTCCGTTGCA								
Ua21	F: TTCCCGAGCTCCAATCACAC	$(TCC)_n$	A	FAM	260–272	7–11	5	3	KY940743
	R: CCATATCCCGTCCTCGCAAA								
Ua22	F: TGGTCCACTTTAGCCAGTCAT	$(ATG)_n$	C	NED	272–290	8–14	6	3	KY940744
	R: TCTGCCCTTGACATCTTTGACA								
Ua23	F: TAGTGCGAGGCCTGATGTTC	$(CTT)_n$	D	PET	244–253	6–9	3	3	KY940745
	R: ACCGAAAAGGCTTGGACGAT								

Note: *A* = number of alleles.

[a]Melting temperature (T_m) for all primers ranged from 59.1°C to 60.3°C.

[b]Tails attached to the forward primers: A = GCCTCCCTCGCGCCA, B = GCCTTGCCAGCCCGC, C = CAGGACCAGGCTACCGTG, D = CGGAGAGCCGAGAGGTG.

[c]The range includes the size of the tail.

TABLE 2. Allelic richness and genetic diversity of SSR loci in four populations of *Usnea* spp. collected on King George Island.[a]

	Total (n = 77)			*U. antarctica* (C1; n = 20)			*U. antarctica* (P3; n = 19)			*U. aurantiacoatra* (C1; n = 20)			*U. aurantiacoatra* (P3; n = 18)		
Locus	n	mean A	H_e	n	A	H_e	n	A	H_e	n	A	H_e	n	A	H_e
Ua1	76	5.3	0.66	20	3	0.36	19	4	0.61	19	6	0.61	18	8	0.90
Ua2	77	5.3	0.75	20	4	0.71	19	5	0.76	20	6	0.76	18	6	0.78
Ua3	77	2.5	0.38	20	2	0.10	19	3	0.37	20	3	0.37	18	2	0.50
Ua4	74	5.5	0.73	20	5	0.72	19	5	0.78	17	5	0.78	18	7	0.77
Ua5	77	1.8	0.08	20	1	0.00	19	2	0.11	20	1	0.11	18	3	0.22
Ua6	77	2.5	0.22	20	2	0.19	19	2	0.28	20	3	0.28	18	3	0.22
Ua7	70	2.3	0.19	20	2	0.19	19	3	0.20	14	2	0.20	17	2	0.22
Ua8	77	2.3	0.16	20	1	0.00	19	1	0.00	20	4	0.00	18	3	0.22
Ua9	76	2.5	0.53	20	2	0.44	19	2	0.46	19	3	0.46	18	3	0.66
Ua10	76	4.5	0.45	19	4	0.30	19	2	0.11	20	5	0.11	18	7	0.75
Ua11	77	4.3	0.68	20	6	0.79	19	5	0.75	20	3	0.75	18	3	0.62
Ua12	76	2.8	0.32	19	1	0.00	19	1	0.00	20	4	0.00	18	5	0.61
Ua13	76	2.8	0.31	20	1	0.00	19	1	0.00	19	4	0.00	18	5	0.56
Ua14	76	4.8	0.73	19	4	0.73	19	6	0.82	20	5	0.82	18	4	0.73
Ua15	77	1.5	0.05	20	1	0.00	19	1	0.00	20	1	0.00	18	3	0.22
Ua16	77	2.5	0.32	20	2	0.10	19	1	0.00	20	4	0.00	18	3	0.66
Ua17	75	3.0	0.54	19	3	0.29	18	3	0.65	20	3	0.65	18	3	0.57
Ua18	77	3.0	0.57	20	4	0.57	19	2	0.49	20	3	0.49	18	3	0.60
Ua19	77	1.5	0.05	20	2	0.10	19	1	0.00	20	1	0.00	18	2	0.11
Ua20	77	2.5	0.32	20	3	0.59	19	3	0.37	20	2	0.37	18	2	0.11
Ua21	77	3.8	0.63	20	4	0.59	19	4	0.59	20	3	0.59	18	4	0.70
Ua22	77	2.0	0.22	20	2	0.19	19	2	0.20	20	1	0.20	18	3	0.50
Ua23	77	1.8	0.21	20	1	0.00	19	1	0.00	20	2	0.00	18	3	0.46
Mean		3.05	0.40		2.61	0.30		2.61	0.33		3.22	0.33		3.78	0.51

Note: A = number of alleles; H_e = Nei's unbiased gene diversity; n = total number of samples.
[a]Locality data for populations can be found in Appendix 1.

on King George Island) were chosen to evaluate the variability of the markers. All samples were tested with multiplexed PCRs of seven to eight markers with the Type-it Multiplex Kit (QIAGEN, Hilden, Germany). PCR reactions were conducted in 10-µL volumes containing 1.5 µg of genomic DNA, 5 µL of PCR Master Mix, and 3 µL of primer multiplex (0.25 µM of each forward primer and fluorescent dye, 0.125 µM of each reverse primer). PCR products were amplified using the same touchdown program as above. PCR amplicons were electrophoresed using an Applied Biosystems 3730 platform, with the LIZ 600 Size Standard (Applied Biosystems, Foster City, California, USA), and allele sizes were manually scored using the Geneious 10 microsatellite tool (Kearse et al., 2012).

The variability of each microsatellite locus was measured by counting the number of alleles and calculating Nei's unbiased gene diversity using GenAlEx 6.5 (Peakall and Smouse, 2012). All of the 23 markers amplified in both species. In *U. aurantiacoatra*, all of the SSR markers were polymorphic, while five were monomorphic in *U. antarctica* (Table 2).

Linkage disequilibrium was tested with GENEPOP (Raymond and Rousset, 1995; Rousset, 2008) twice: separately for each species to estimate P values and with the two species together to perform a global test (Fisher's method) for each pair of loci across populations and species. Holm-Bonferroni correction (Holm, 1979) of the P values resulting from the last test using the implementation by Gaetano (2013) revealed significant evidence for linkage disequilibrium between loci Ua3, Ua4, and Ua6 (Ua3–Ua4: P = 0.001, Ua3–Ua6: P = 0.006, Ua4–Ua6: P = 0.0).

Cross-species amplification was tested on DNA extracts of *U. sphacelata* R. Br. and *U. trachycarpa*, two related species from *Usnea* subgenus *Neuropogon* (Nees & Flotow) Jatta (Truong et al., 2013). First, we tested a population of eight samples of *U. sphacelata* with the multiplex protocol. To verify the identity and stability of the flanking regions, we then selected two samples with different peaks for single PCR amplification and sequencing. The multiplex analysis was omitted for *U. trachycarpa.* Thirteen markers showed the same flanking regions in all three species, five markers had deletions and/or insertions in the flanking regions in *U. sphacelata* and *U. trachycarpa*, and for four markers the PCRs failed. Marker Ua18 shared the flanking region only with *U. sphacelata*, while it had a deletion in *U. trachycarpa* (Table 3).

CONCLUSIONS

The markers developed here are suitable to study population structure and gene flow in *U. antarctica* and *U. aurantiacoatra*. The markers shared the same flanking regions in the two species and showed high variability even within a small geographic area. Nineteen of these microsatellite primers also cross-amplify in related species from *Usnea* subgenus *Neuropogon*; however, the flanking regions of some markers are less stable. The usability of the newly developed SSR markers for cross-amplification in other *Usnea* species therefore requires further validation using a broader taxon sampling and a higher number of replicates per species. We conclude that the newly developed SSR markers presented here can be used to infer gene flow within Southern Hemisphere lichen populations and resolve connectivity patterns among populations of *U. antarctica* and *U. aurantiacoatra*.

TABLE 3. Cross-amplification of SSR markers developed for *Usnea antarctica* and *U. aurantiacoatra* with related species of *Usnea* subgenus *Neuropogon.*

Species	Ua1	Ua2	Ua3	Ua4	Ua5	Ua6	Ua7	Ua8	Ua9	Ua10	Ua11	Ua12	Ua13	Ua14	Ua15	Ua16	Ua17	Ua18	Ua19	Ua20	Ua21	Ua22	Ua23
U. sphacelata	+	—	+	+	D	D	+	—	—	+	+	+	+	—	+	D	+	+	+	D	+	D	+
U. trachycarpa	+	—	+	+	D	D	+	—	—	+	+	+	+	—	+	D	+	D	+	D	+	D	+

Note: + = SSRs present; — = PCR failed; D = deletion/insertion within the flanking region.

LITERATURE CITED

Blacket, M. J., C. Robin, R. T. Good, S. F. Lee, and A. D. Miller. 2012. Universal primers for fluorescent labelling of PCR fragments—An efficient and cost-effective approach to genotyping by fluorescence. *Molecular Ecology Resources* 12: 456–463.

Bolger, A. M., M. Lohse, and B. Usadel. 2014. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* 30: 2114–2120.

Cubero, O. F., and A. Crespo. 2002. Isolation of nucleic acids from lichens. *In* I. C. Kranner, R. P. Beckett, and A. K. Varma [eds.], Protocols in lichenology: Culturing, biochemistry, ecophysiology, and use in biomonitoring, 381–391. Springer, Berlin, Germany.

Gaetano, J. 2013. Holm-Bonferroni sequential correction: An Excel calculator (1.2). Website https://www.researchgate.net/publication/242331583_Holm-Bonferroni_Sequential_Correction_An_EXCEL_Calculator_-_Ver._1.2 [accessed 8 June 2017].

Holm, S. 1979. A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics* 6: 65–70.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, et al. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649.

Nurk, S., A. Bankevich, D. Antipov, A. A. Gurevich, A. Korobeynikov, A. Lapidus, A. D. Prjibelski, et al. 2013. Assembling genomes and mini-metagenomes from highly chimeric reads. *In* M. Deng, R. Jiang, F. Sun, and X. Zhang [eds.], Research in computational molecular biology, vol. 7821, 158–170. Springer, Berlin, Germany.

Øvstedal, D. O., and R. I. Lewis Smith. 2001. Lichens of Antarctica and South Georgia. Studies in Polar Research. Cambridge University Press, Cambridge, United Kingdom.

Peakall, R., and P. E. Smouse. 2012. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research–An update. *Bioinformatics* 28: 2537–2539.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86: 248–249.

Rousset, F. 2008. GENEPOP'007: A complete reimplementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* 8: 103–106.

Strous, M., B. Kraft, R. Bisdorf, and H. E. Tegetmeyer. 2012. The binning of metagenomic contigs for microbial physiology of mixed cultures. *Frontiers in Microbiology* 3: 410.

Thiel, T., W. Michalek, R. K. Varshney, and A. Graner. 2003. Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). *Theoretical and Applied Genetics* 106: 411–422.

Truong, C., P. K. Divakar, R. Yahr, A. Crespo, and P. Clerc. 2013. Testing the use of ITS rDNA and protein-coding genes in the generic and species delimitation of the lichen genus *Usnea* (Parmeliaceae, Ascomycota). *Molecular Phylogenetics and Evolution* 68: 357–372.

Wirtz, N., C. Printzen, and H. T. Lumbsch. 2012. Using haplotype networks, estimation of gene flow and phenotypic characters to understand species delimitation in fungi of a predominantly Antarctic *Usnea* group (Ascomycota, Parmeliaceae). *Organisms Diversity and Evolution* 12: 17–37.

Zhang, J., K. Kobert, T. Flouri, and A. Stamatakis. 2014. PEAR: A fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* 30: 614–620.

Appendix 1. Locality and collection data for *Usnea* samples used in this study.

Species (Population)	*n*	Geographic coordinates	Locality data	Collector (Collection year)	Voucher no. (Herbarium)[a]
U. antarctica Du Rietz (C1)	20	62°14.220′S, 58°39.492′W	King George Island, Antarctica	Lagostina, Kanz (2015)	FR-0264579 (FR)
U. antarctica (P3)	19	62°15.174′S, 58°38.971′W	King George Island, Antarctica	Lagostina, Kanz (2015)	FR-0264581 (FR)
U. aurantiacoatra (Jacq.) Bory (C1)	20	62°14.220′S, 58°39.492′W	King George Island, Antarctica	Lagostina, Kanz (2015)	FR-0264584 (FR)
U. aurantiacoatra (P3)	18	62°15.174′S, 58°38.971′W	King George Island, Antarctica	Lagostina, Kanz (2015)	FR-0264586 (FR)
U. aurantiacoatra	1	62°14.363′S, 58°39.202′W	King George Island, Antarctica	Lagostina, Kanz (2015)	FR-0264585 (FR)
U. sphacelata R. Br.	4	79°17′N, 16°04′W	Spitsbergen, Svalbard	Tønsberg (2002)	BG-L94105 (BG)
U. sphacelata	4	64°45′N, 18°15′W	Central Highland, Iceland	Heidmarsson (2002)	Heidmarsson 28916 (F)
U. trachycarpa (Stirt.) Müll. Arg.	2	54°58.257′S, 67°38.004′W	Isla Navarino, Chile	Printzen (2017)	FR-0264590 (FR)

Note: *n* = number of individuals tested.

[a]Vouchers are deposited in the Herbarium Senckenbergianum Frankfurt (FR), Frankfurt am Main, Germany; University Museum of Bergen (BG), Bergen, Norway; and the Field Museum of Natural History (F), Chicago, Illinois, USA.